suggests instead that it is a decrease in ribosome biogenesis that accounts for the increase in replicative life span upon deletion of *SCH9* or *TOR1* (Kaeberlein et al., 2005). These studies suggest an intriguing model in which similar nutrient-responsive signaling pathways coordinate aging in mitotic and postmitotic cells, albeit through different downstream effectors (Figure 1B).

A long-standing and still unresolved debate among biogerontologists concerns whether the aging process is controlled by a relatively small number of regulatory pathways or whether aging results from many different and cell-type-specific changes that occur over time. Certainly different types of cells show different aging characteristics. On the other hand, single gene mutations can profoundly delay the rate of aging in all of the well-studied model systems, including mice and rats. A network in which regulatory proteins are used to coordinate a wide range of downstream events important to aging could unite both of these models and may help to explain the wide range of aging phenotypes retarded by calorie restriction. The combined use of both chronological and replicative models of yeast aging will continue to be a powerful approach for dissecting the molecular mechanisms that coordinate longevity.

Brian K. Kennedy,[1] Erica D. Smith,[1] and Matt Kaeberlein[2]
[1]Department of Biochemistry
[2]Departments of Genome Sciences and Medicine
University of Washington
Seattle, Washington 98195

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A Nose by Any Other Name (Should Smell as Sweetly)

The standard view that the control of mating behavior by pheromones is mediated by the vomeronasal organ, and not by the main olfactory epithelium, has recently been called into question. In this issue of *Cell*, two independent studies (Boehm et al., 2005; Yoon et al., 2005) examine the inputs from each of these olfactory pathways to a population of neurons that plays a central role in mating behavior.

How many of us have chosen our mates because of an ineffable, unconscious sensory attraction that we call "chemistry?" We intuit that such chemistry may be olfactory, or "pheromonal," but evidence for sexual pheromones in humans is limited. In lower organisms, mating pheromones have been thought to be detected by the vomeronasal organ (VNO), whereas the main olfactory epithelium (MOE), the "conscious nose," detects general odorants. However, this dichotomous detection system cannot explain pheromonal attraction in humans, if it indeed exists, because we lack a VNO. Recent studies (Lin et al., 2005; Mandiyan et al., 2005), including two elegant papers in this issue of *Cell* (Boehm et al., 2005; Yoon et al., 2005), may help to clarify this conundrum.

The MOE and VNO engage distinct circuits within the brain, as shown by classical neuroanatomical tracing studies (Figure 1). A simplified picture is that sensory neurons in the MOE project to the main olfactory bulb, which then relays information to the olfactory cortex and cortical amygdala (Figure 1). In contrast, sensory neurons in the VNO project to the accessory olfactory bulb, which then projects to nuclei in the medial amygdala (Figure 1). Neurons in the medial amygdala then relay information to the hypothalamus, which coordinates appropriate behavioral and endocrine responses.

Although the amygdala-hypothalamic core pathway for reproductive behavior is likely to be "hard wired," (Choi et al., 2005), its functions are regulated by neuromodulators such as hormones and neuropeptides. In particular, reproductive endocrine status is controlled by the decapeptide gonadotropin-releasing hormone (GnRH, also called LHRH or luteinizing hormone-releasing hormone). This peptide controls the production of gonadal steroids, by regulating the release of luteinizing hormone (LH) and follicle stimulating hormone from the anterior pituitary. However, GnRH/LHRH neurons may regulate other aspects of reproductive behavior as well (Meredith, 1998). Remarkably, these influences are mediated by a population of only ~800 GnRH/LHRH neurons, that are present in the medial preoptic area of the hypothalamus and basal forebrain, but also scattered throughout several other brain regions (the yellow dots in Figure 1).

Previous work has suggested that GnRH neuronal activity is controlled by VNO-mediated chemosensory input. Removal of the VNO in rodents blocks mating-induced increases in serum LH levels. VNO removal also suppresses mating-induced expression of the immediate-early gene c-*fos*, a marker of neuronal activa-

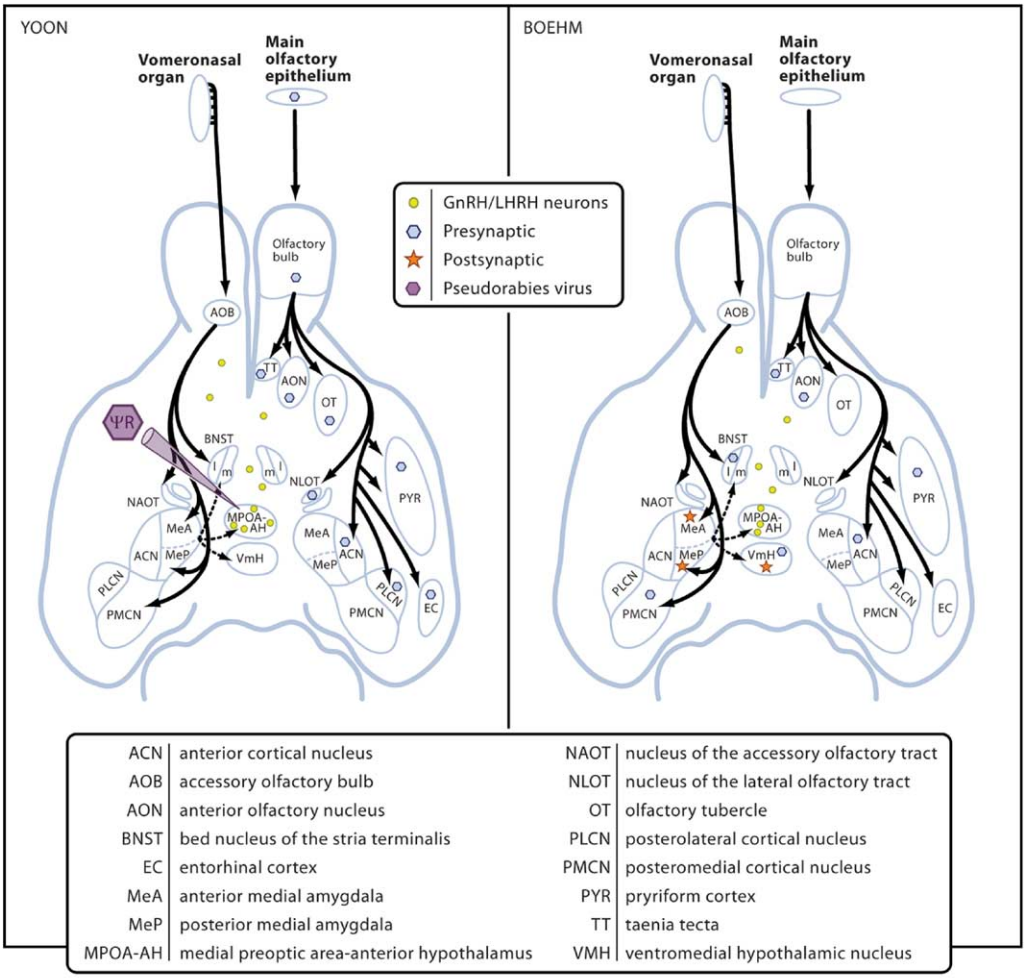

Figure 1. Similarities and Differences between Yoon and Boehm Studies

This figure illustrates the similarities and differences in the results between Yoon et al. (2005) (left panel) and Boehm et al. (2005) (right panel), superimposed on a diagram representing the vomeronasal and main olfactory pathways. For clarity, these pathways are represented on the left and right sides of the horizontal brain diagrams, respectively, and are not meant to imply bilateral asymmetry or lack of convergence between them. Yoon et al., (2005) (left panel) generated a mouse line containing a 212 kb BAC *GnRH/LHRH* transgene, from which Cre recombinase is expressed. The brains of these mice were stereotaxically injected in the medial preoptic area, medial septum and several other locations, with a GFP-expressing pseudorabies virus (ΨR), Ba2001, which only replicates in Cre-expressing neurons. Boehm et al. (2005) (right panel) generated a transgenic mouse in which a ~3.5 kb 5′ GnRH enhancer fragment drives the expression of barley lection (BL), a transneuronal tracer. In both panels, small yellow dots represent cell bodies of GnRH/LHRH neurons; blue hexagons represent neurons presynaptic to GnRH/LHRH neurons. Orange stars (right panel only) represent neurons postsynaptic to GnRH/LHRH neurons. Not all regions projecting to, or receiving projections from, GnRH/LHRH neurons are shown. Yoon et al. (2005) (left panel) observe presynaptic inputs to GnRH/LHRH neurons only in the main olfactory pathway, whereas Boehm et al. (2005) (right panel) observe inputs in both the main and vomeronasal pathways. Note that Yoon et al. (2005) measures inputs only to GnRH/LHRH neurons present at sites of pseudorabies virus (ΨR) injection (left panel), whereas Boehm et al. (2005) (right panel) measure inputs to all ~800 GnRH/LHRH neurons. The drawing is modified from Meredith (1998) and Figure 4 of Yoon et al. (2005).

tion, in GnRH neurons. Conversely, electrical stimulation of the VNO activates c-*fos* expression. Finally, deficits in reproductive behaviors caused by VNO lesions can be restored by intracerebral administration of GnRH (Meredith, 1998). Consistent with these functional studies, axonal tracing indicates that the vomeronasal pathway projects to the medial preoptic area, where many GnRH/LHRH neurons are located.

To gain more insight into the circuits controlling reproductive status and behavior, the authors of two papers in this issue of *Cell* (Boehm et al., 2005; Yoon et al., 2005) directly investigated the olfactory pathways that relay sensory input to GnRH/LHRH neurons. To do this, they targeted trans-neuronal tracers to GnRH/LHRH neurons in transgenic mice, and followed the movement of these tracers in the brain. The results of both studies indicate that, at the very least, the VNO may not be the sole source of input to GnRH neurons, and one of the studies (Yoon et al., 2005) suggests, surprisingly, that this input may be negligible.

Buck and colleagues (Boehm et al., 2005) generated a transgenic mouse in which a ~3.5 kb 5′ GnRH enhancer fragment drives the expression of barley lection (BL), a transneuronal tracer. Strikingly, the authors

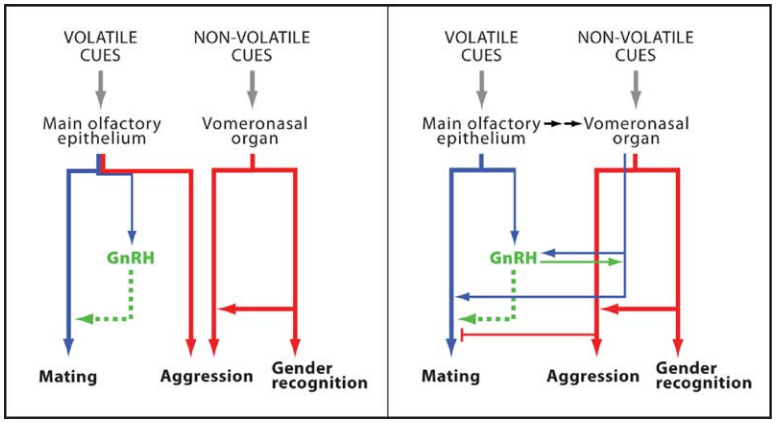

Figure 2. Possible Roles of the Main Olfactory and Vomeronasal Pathways in Reproductive and Defensive Behaviors

Simplified circuit diagrams illustrate the possible roles of the main olfactory and vomeronasal pathways in reproductive (blue) and defensive (red) behaviors. Thick arrows indicate functional requirement based on inactivation experiments; thin arrows indicate connectivity (direct or indirect) based on tracing and/or c-*fos*/MAPK activation experiments. Dashed arrow (green) indicates that GnRH neurons may play a direct, essential role, or a modulatory function, in mating behavior. In the left panel, the MOE acts independently of the VNO to control mating behavior. In the right panel, the VNO plays a redundant role with the MOE in mating behavior. In the left panel, the MOE acts in a nonredundant manner with the VNO to control aggression, whereas in the right panel it acts in series (black arrows) with the VNO to promote aggression, by controlling access to nonvolatile odorants (Mandiyan et al., 2005). These alternative aggression circuits are illustrated arbitrarily in the left and right panels, and could be reversed. The blunt line indicates the cross-inhibition of reproductive circuits by defensive circuits that is proposed by Choi et al. (2005). Intermediate stages in the circuit such as the accessory olfactory bulb, the main olfactory bulb, amygdala and hypothalamus are omitted for simplicity. Arrows are not meant to indicate excitatory synapses, but rather indicate the net effect of pathway activation.

found that the GnRH neurons connected with ~50,000 neurons in 53 different brain regions. Because BL travels in both anterograde (forward) and retrograde (backward) directions, BL^+ cells will comprise: (1) retrogradely labeled neurons presynaptic to GnRH-BL-expressing cells (Figure 1, right panel, blue hexagons); (2) anterogradely labeled neurons postsynaptic to GnRH-BL-expressing cells (Figure 1, right panel, orange stars); and (3) GnRH-BL-expressing neurons themselves (Figure 1, right panel, yellow dots). To distinguish these alternatives, the authors stained adjacent sections with antibodies to GnRH, which labels cell bodies and axons, but not the dendrites, of GnRH neurons. The authors assumed that any BL^+ cells present in regions devoid of GnRH fiber or cell body staining were retrogradely labeled. Presynaptic BL^+ neurons were found in the vomeronasal pathway (Figure 1, right panel blue hexagons), and some of these were also activated by pheromonal stimuli, as revealed by c-*fos* colabeling. These data are consistent with the prevailing view that GnRH neurons receive pheromonal information from the VNO. However, the authors also detected presynaptic BL^+ neurons in the main olfactory pathway (Figure 1, right panel, blue hexagons), suggesting that some GnRH neurons may also receive olfactory input from the MOE. Whether the same or different GnRH neurons receive these dual inputs is not yet clear.

Dulac and colleagues (Yoon et al., 2005) used a different approach, generating a mouse line containing a 212 kb BAC *GnRH/LHRH* transgene, from which Cre recombinase is expressed. The brains of these mice were stereotaxically injected in the medial preoptic area, medial septum and several other locations, with a pseudorabies virus expressing GFP (Ba2001). This virus only replicates in neurons that express Cre (Figure 1, pseudorabies virus is indicated by ΨR). This method differs in several respects from that of Boehm et al. (2005): First, propagation and transfer of Ba2001 take place only in the retrograde direction; second, transport of the tracer occurs only from those GnRH/LHRH neurons at the site of viral injection, rather than from every GnRH/LHRH cell in the brain; third, labeling by the tracer changes with time after injection, whereas the labeling in *GnRH-BL* mice reflects steady-state expression of the transgene; fourth, neurons infected with the pseudorabies viral tracer degenerate after about a week, due to cytotoxicity, whereas there is no evidence that BL causes such toxicity.

Like Boehm et al. (2005), Dulac and colleagues detected retrogradely labeled GFP^+ cells in the main olfactory pathway, in this case following the tracer all the way to sensory neurons in the MOE (Figure 1, left panel, blue hexagons). Surprisingly, however, and unlike Boehm et al. (2005), they found no labeling in the vomeronasal pathway (Figure 1, left panel). Positive control experiments, using a nonconditional pseudorabies virus, demonstrated that the vomeronasal pathway could indeed be labeled by injection into the medial preoptic area. What explains the discrepancy between these two datasets? In addition to the substantial methodological differences described above, differences in the *GnRH/LHRH* promoters used may contribute as well. Yoon et al. (2005) also acknowledge that they cannot exclude the possibility that synaptic input from VNO to GnRH/LHRH neurons might be resistant to Ba2001 transfer. Alternatively, the presynaptic inputs from the VNO pathway identified by Boehm et al. (2005) might derive from a subset of GNRH/LHRH neurons distinct from those targeted by Yoon et al. (2005).

It is important to point out that the observations of Yoon et al. (2005) do not refute previous neuroanatomical tracing data indicating that the vomeronasal pathway projects to the hypothalamic area where GnRH/LHRH neurons are located. GnRH/LHRH neurons constitute only a subpopulation in this area, and the control virus injections confirm that other neurons in this area do indeed receive projections from the vomeronasal pathway. Yet it remains difficult to reconcile the conclusion that GnRH/LHRH neurons receive no input from the vomeronasal pathway, with earlier data indicating that VNO input is necessary and sufficient for activation of these neurons. Yoon et al. (2005) suggest that this

activation could be mediated by nonsynaptic (e.g., hormonal or neuromodulatory) mechanisms.

Because the most surprising result of their tracing study is based on negative data, Yoon et al. (2005) turned to functional studies. They reasoned that if GnRH/LHRH neurons are indeed important for reproductive behavior, and if these neurons receive their primary input from the main olfactory pathway, then the MOE should be required for reproductive behavior. To test this directly, they generated mice in which the MOE, but not the VNO, was genetically inactivated by mutation of the cyclic nucleotide-gated channel α (CNG$\alpha^{-/-}$), or inactivated chemically. These mice failed to exhibit a characteristic increase in the expression of phosphorylated MAPK (another marker of neuronal activation) upon exposure to reproductive olfactory stimuli. They also exhibited a dramatic reduction in chemosensory investigation of females, as well as a reduction in the number of mounting attempts. Independent work by Mandiyan et al. (2005) also reports defects in mating behavior and investigation of female odors in male CNG$\alpha^{-/-}$ mice.

Together, these studies suggest an essential role for the MOE in mating behavior (Figure 2, blue arrows). By contrast, previous studies indicated that mice with a knockout in *TrpC2*, which is required for VNO but not MOE function, show no defects in male-female mating (Leypold et al., 2002; Stowers et al., 2002), as do those with surgical VNO lesions (Pankevich et al., 2004). These data do not, however, exclude the possibility that the VNO plays a redundant role in some aspects of mating, which can be compensated by the MOE (Figure 2, right panel, blue arrows). Nor do they exclude a role in mating for indirect targets of the VNO. For example, another recent tracing study (Choi et al., 2005) reported that in male mice, female urine induces c-*fos* in Lhx6^{+} neurons in the medial amygdala that project to reproductive hypothalamic nuclei, a classical segment of the vomeronasal pathway. Whether this activation is VNO-dependent remains to be determined.

Where, then, does that leave the role of the diminutive VNO? Behavioral analysis of *TrpC2*$^{-/-}$ mice has indicated a requirement for the VNO in gender recognition and/or aggression (Leypold et al., 2002; Stowers et al., 2002). Surprisingly, Mandiyan et al. (2005) also observe defects in inter-male aggression in mice lacking CNGα. One interpretation of this observation is that the MOE and VNO each play nonredundant, essential roles in aggression (Figure 2, left panel, red arrows). Another is that the VNO alone is essential for aggression, but that the MOE is indirectly required for VNO function (Figure 2, right panel, black arrows). In support of the second explanation, Mandiyan et al. (2005) point out that detection of nonvolatile pheromones by the VNO requires sniffing behavior (Luo et al., 2003), which is strongly reduced in MOE-defective mice.

The finding that the main olfactory pathway is required for reproductive behavior, and that it projects to and activates GnRH/LHRH neurons, is exciting. However, these data do not yet distinguish whether these neurons are directly required for mating behavior, or if they modulate it (Figure 2). To answer this question, temporally controlled ablation or silencing of GnRH neurons will be necessary. Conditional knockout of GnRH/LHRH itself should distinguish, similarly, whether this peptide is an essential component or a modulator of mating behavior. It will also be important to reconcile the requirement of the MOE for mating, with a similar requirement for medial amygdala nuclei that receive projections from the VNO. Electrophysiological data suggests that nuclei of the medial amygdala also receive input from the main olfactory cortex. Thus, these regions offer a potential site of integration of VNO and MOE input (Licht and Meredith, 1987). The results of Boehm et al. (2005), Yoon et al. (2005), and Mandiyan et al. (2005), taken together with the ability of the MOE to detect pheromones (Lin et al., 2005), may explain how organisms without a VNO, such as ourselves, use olfactory inputs to activate subcortical, hard-wired circuits for innate reproductive behavior.

Gloria B. Choi[1] and David J. Anderson[1,2]
[1]Division of Biology 216-76
California Institute of Technology
[2]Howard Hughes Medical Institute
Pasadena, California 91125

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